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Specificity and stability in high latitude eastern Pacific coral–algal symbioses

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Abstract

The Gulf of California (Sea of Cortez) acts as a refuge for zooxanthellate coral communities at high northern latitudes in the eastern Pacific. The diversity of dinoflagellate endosymbionts living with cnidarians (9 genera, 16 species) was examined using denaturing gradient gel electrophoresis (PCR-DGGE) fingerprint analysis of the rDNA Internal Transcribed Spacers (ITS) 1 and 2. Although geographically isolated from the rest of the Pacific, coral–algal associations in the Gulf of California were similar in cladal composition (dominated by clade C *Symbiodinium*). However, levels of host–symbiont specificity appeared to be greater than in the western Pacific, where host diversity is considerably higher and environmental conditions are relatively stable. As a result, the cnidarian community lacked a host–generalist symbiont. Most colonies of the ecologically dominant genus *Pocillopora* contained uniform populations of either *Symbiodinium* D1 (in ~70% of colonies) or C1b-c (~ 30%). The proportions of D versus C colonies (i.e., D and C holobionts) remained stable between the years 2004 and 2006. The specificity, stability, and prevalence of *Symbiodinium* D1 found among individuals of *Pocillopora* spp. in a region minimally impacted by coral bleaching indicate that these symbiotic combinations, exhibiting specific variability, appear to be products of long-term ecological and evolutionary processes.

Biogeographic patterns provide important information used to infer ecological and evolutionary processes. Many questions remain unanswered with regard to the symbiosis ecology of corals possessing obligate associations with intracellular dinoflagellates (zooxanthellae in the genus *Symbiodinium*). The detailed analysis of the geographic and host-habitat distributions of the microbial eukaryote partner enables us to evaluate the effect of isolation, environment, and host biology on the evolutionary processes that create different host–symbiont combinations (Rowan and Powers 1991; Baker 2003; LaJeunesse 2004). From the perspective of ongoing global climate change, comparisons between remote regions with differing environmental conditions provide a realistic measure of the extent to which these associations change with time.

Symbiodinium spp. diversity and patterns of host–symbiont specificity are known for only a few regions in the Pacific. By comparison, virtually nothing is known about coral symbioses in the eastern Pacific (Glynn et al. 2001). Greater seasonal fluctuations in temperature, turbidity, and nutrients, as well as geographic isolation, have impoverished the assemblages of coral species in this region. The coral community comprises mostly Indo-Pacific migrants (Reyes Bonilla 2002) that periodically reach the east by long distance dispersal. Several endemics have evolved since the Pliocene closure of the Central American isthmus (e.g., *Porites arnaudi* and *Pocillopora effuses*; Veron 2000), and a few are probably relic species from west Atlantic ancestral

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stocks (e.g., *Siderastrea glynni*; Forsman et al. 2005). This collection of Pliocene relics, new endemics, and long distance migrants, therefore, constitutes a separate and unique Pacific faunal province (Veron 1995). The unusual host assemblages and marginal environmental conditions present in this isolated region are of vital interest to the general study of cnidarian-dinoflagellate evolution and ecology.

The effect of strong El Niño Southern Oscillation (ENSO) events on corals and their endosymbionts is another important reason for studying these communities. In the 1980s and 1990s mass coral bleaching and mortality negatively affected many eastern Pacific coral communities (Glynn 1990). "Coral bleaching" refers to the rapid dissociation of host and pigmented symbiont in response to stress, such as high temperature and irradiance (c.f. Fitt et al. 2001; Coles and Brown 2003). After severe episodes of stress, many coastal regions in Panama, Ecuador, and Mexico had severe reductions in their coral populations (Glynn 1990; Reyes-Bonilla et al. 2002). Not all eastern Pacific communities, however, experienced these losses.

One of the least affected areas has been the Gulf of California. Whereas regions in southwestern Mexico (and elsewhere) suffered severe bleaching and mortality during the 1997–1998 ENSO, areas inside the Gulf of California were largely spared (Reyes Bonilla 2001; Reyes Bonilla et al. 2002). This Gulf, also known as the Sea of Cortez, is relatively isolated from Central and South American reef systems. At the mouth of the sea, the California Current and the Costa Rica Coastal Current collide to create an oceanographic front that acts as a hydrographic barrier limiting larval dispersal into and out of this inner sea (Fiedler 2002). This barrier partially explains why the Gulf of California has been considered a separate zoogeographical province, the Cortezian Province, and also why coral composition in the Gulf is different from that of tropical Mexico (Reyes Bonilla 2002). During past ENSO events, this hydrology diminished the northern advance of warm water masses transported from the west Pacific, which reduced the thermal stress and associated negative ecological effects to coral communities in the Gulf.

The study of coral symbioses in high latitude regions of the eastern Pacific provides important ecological and biogeographic perspectives on the stability and variability of coral–algal symbioses. PCR-DGGE of the ribosomal DNA (rDNA) internal transcribed spacers (ITS) 1 and the ITS 2 were conducted on populations of *Symbiodinium* spp. that were sampled from cnidarian host communities, including stony corals, anemones, and zoanthids. These data illustrate that, despite wide seasonal fluctuations in light and temperature, coral–algal symbioses are highly specific and stable. Observations on cnidarian–*Symbiodinium* relationships from less disturbed eastern Pacific regions, like the Sea of Cortez, must be considered when explaining the ecological response of coral symbioses to thermal disturbances (Baker et al. 2004).

Materials and methods

Collections—In early May 2004, symbiotic cnidarians from the intertidal zone to a depth of approximately 10–12 m were sampled at locations extending across 80 km of coastline near La Paz, Mexico (Fig. 1). Water clarity in the Eastern Pacific limits the maximum depth distributions of most zooxanthellate cnidarians to about 10 m or 15 m (Glynn and Ault 2000). Branch fragments of 4 cm were taken from average-sized colonies of *Pocillopora* spp., whereas pieces of approximately 4–5 cm^2 where chiseled from massive-shaped species. To avoid sampling from the same clone (ramet) of *Pocillopora* spp., colonies with distinctive morphologies were chosen every 5 m to 10 m while swimming parallel to the shoreline. Whole anemones and zoanthids were scraped off their substrate. Skeletal fragments of scleractinians were stripped of tissue using an airbrush and the blastate processed as described by LaJeunesse et al. (2003). Algal pellets were transferred to 1.5 mL microcentrifuge tubes and preserved in 20% dimethylsulfoxide (DMSO), 0.25 mol L^{-1} ethylenediamenetetraacetic acid (EDTA), in sodium chloride-saturated water solution (Seutin et al. 1991). Oral discs were removed and homogenized from soft-bodied actiniarian and zoanthidian taxa, and the *Symbiodinium* cells separated by centrifugation as described by LaJeunesse (2002).

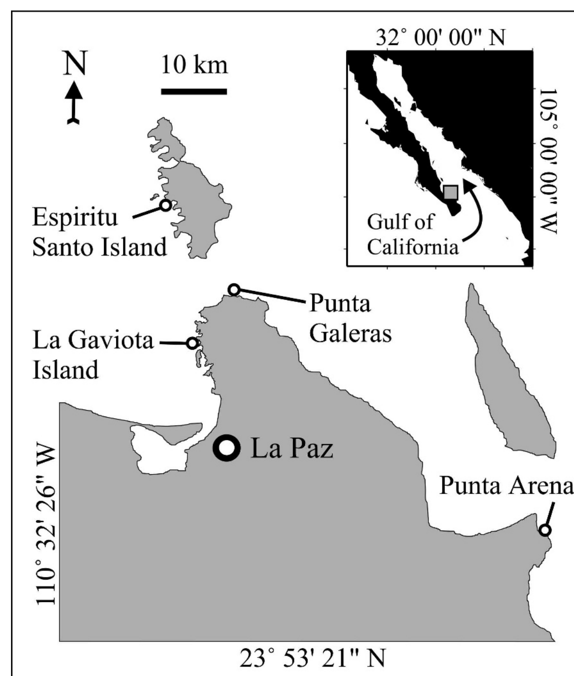

Fig. 1. Sampling locations around La Paz, Mexico, inside the southern Gulf of California or Sea of Cortez (inset).

Sampling from 2004 showed that *Pocillopora* spp. were associated with two species of *Symbiodinium* (*see* Results). To examine the distribution of these symbioses further, 18 to 24 colonies of morphologically distinctive *Pocillopora* spp. were tagged and sampled along each of three transects paralleling the shoreline at Punta Galeras (24°21′15.4″N, 110°17′05″W) and along each of three transects on the south side of La Gaviota Island (24°17′12′N, 110°20′20′W; Fig. 1) in early May, 2006. Three random colonies were selected from each transect (n = 18) and sampled extensively at the tips and bases of branches taken from

the east, west, north, south, and center regions of the colony.

Artificial combinations of the two different symbiont species were created from fresh isolations to test the sensitivity of PCR-DGGE. In August 2006, samples from tagged colonies, whose symbiont populations were previously analyzed and found to be homogenous based on collections from May 2006, were collected. Homogeneity was confirmed using real-time PCR analysis optimized to distinguish the minority species to levels at least 0.05% of the total population (LaJeunesse et al. 2007). Cell ratios were determined from five replicate cell counts using a hemocytometer.

DNA extractions, PCR-DGGE, and sequencing—Nucleic acid extractions on 10 mg to 30 mg of dinoflagellate material were conducted using a modified Promega Wizard genomic DNA extraction protocol (LaJeunesse et al. 2003). The ITS regions 1 and 2 were amplified from each extract using the respective primer sets ITS 1 clamp (CGCCC-GCCGC GCCCCGCGCC CGTCCCGCCG CCCCCG-CCC GGGATCCGTT TCCGTAGGTG AACCTGC) and ITSintrev2 (TTC ACG GAG TTC TGC AAT); and ITS 2 clamp and ITSintfor2 (LaJeunesse and Trench 2000) with the touch-down thermal cycle as described in LaJeunesse et al. (2003). Products from these PCR reactions were electrophoresed on denaturing gradient gels (45–80%) using a CBScientific system.

The production of PCR-DGGE generated fingerprints, followed by the excision and sequencing of the most diagnostic (brightest) and lowest bands, introduces a critical step for filtering out uninformative variants that are distributed throughout the ribosomal array of a genome at low copy number. Bands characterizing a consistent fingerprint profile were excised, re-amplified, and directly sequenced as explained by LaJeunesse (2002) and LaJeunesse et al. (2003). The PCR-DGGE protocol for the analysis of rDNA reveals: (1) whether a genome is represented by a single numerically dominant sequence (one prominent band), or (2) that co-dominant variants exist within the genome (multiple bands creating a consistent PCR-DGGE fingerprint profile), and finally (3) that multiple symbionts are present in the host (the mixture of two or more known fingerprints in one lane; LaJeunesse 2002; Thornhill et al. 2006).

Phylogenetic analyses—Sequences were aligned manually using Sequence Navigator version 1.0 software (ABI, Division of Perkin Elmer). *Symbiodinium kawagutii* from clade F was used as an out-group. Base substitutions in ITS rDNA in *Symbiodinium* clade C are far from saturation (LaJeunesse 2004), therefore, maximum parsimony analyses (under the DELTRAN setting using the heuristic search mode) were conducted using PAUP* (Swofford 2000) to produce phylogenetic reconstructions. Maximum parsimony enables the use of informative sequence gaps and insertions (one entire indel is scored as a 5th character state). Applications of neighbor-joining, maximum likelihood, and Bayesian inference methods, which do not take into account indels, have consistently yielded similar phylogentic reconstructions for these datasets (LaJeunesse 2004).

Results

PCR-DGGE of the ITS 1 and 2 diversity of Symbiodinium *in the Sea of Cortez*—*Symbiodinium* diversity was determined in 284 specimens taken from 16 host species in 9 genera (Table 1). Six of these genera associated specifically with one out of the 13 *Symbiodinium* spp. characterized by PCR-DGGE ITS 2 fingerprinting (1 clade A, 1 clade B, 10 clade C, and 1 clade D) (Fig. 2A). PCR-DGGE analysis targeting the ITS 1 resolved eight clearly distinguishable types (1 clade A, 1 clade B [not shown], 5 clade C, and 1 clade D; Fig. 2B), and no further diversity was identified using the ITS 1 that had not already been characterized using the ITS 2. Six of the *Symbiodinium* spp. characterized from the Gulf of California occur in other regions of the Pacific (e.g., C1f in Hawaii; Fig. 2C)

The difference in resolution between the ITS 1 and ITS 2 was that closely-related clade C *Symbiodinium*, C1b-c, C1c, C1f, and C1o, associated with *Pocillopora, Pavona, Psammocora*, and *Protopalythoa,* respectively, were not well resolved by the ITS 1 (Fig. 2B). Comparison of ITS 1 PCR-DGGE profiles, however, showed consistent differences in faint and/or slightly blurred bands that supported the ITS 2 differentiation (not shown). The bands that define the C66 and C1 ITS 2 fingerprints co-migrated to the extent that, unless run side by side, were difficult to distinguish (Fig. 2A). The ITS 1 fingerprints, however, were clearly different between C1 and C66 (Fig. 2B) indicating that the combined analyses of both spacer regions can reduce the potential for errors in symbiont identification.

Clade C phylogenetic reconstructions based on the sequences of the diagnostic bands from PCR-DGGE ITS 1 and 2 fingerprints were generated using maximum parsimony (Fig 3A,B). A comparison of each phylogeny reveals overall similarity, however, phylogenetic resolution differed (explained above). The ITS 1 phylogram had fewer terminal nodes than the ITS 2, yet longer branch lengths (more sequence changes) separated C1 from C66 and C75 (Fig. 3B). Analyses of the concatenated ITS 1 and ITS 2 sequences produced a deeply branched phylogeny (Fig. 3C).

*Variation in coral–*Symbiodinium *spp. combinations: holobiont diversity*—Two divergent *Symbiodinium* spp. (C1b-c and D1) associated uniquely with species of *Pocillopora* (Table 1). Most colonies appeared to possess populations dominated by either C1b-c or D1. Ten independent samples taken from each of 17 out of 18 colonies showed no differences in the symbiont profile (Fig. 4). The upper and lower portions from a west-facing branch contained detectable mixtures of the two symbionts in only one colony (not shown). These findings indicate that a single sample taken from a colony is usually indicative of which symbiont species dominates the entire colony.

Biased PCR amplifications may have artificially lowered the percent of colonies identified with mixtures of both

Table 1. Taxonomic list of host specimens and their identified symbiont "type." The symbiont alphanumeric identifies the clade (uppercase letter), ITS type (number), and presence of any diagnostic co-dominant variant that makes its PCR-DGGE fingerprint distinctive from others (lowercase letter). "Type" designations separated by a forward slash were identified as mixtures in the same sample. Numerals in parentheses represent the quantity of individual colonies found to have a particular symbiont. These data were pooled from collections made at four locations in the southern Gulf of California (Fig. 1).

Host order and family	Host genus and species	Depth of collection	*Symbiodinium* type
Scleractinia			
Agariciidae			
	Pavona clavus	4–7 m	C1c (5)
	Pavona gigantea	3–12 m	C1c (14)
Pocilloporidae			
	Pocillopora verrucosa	1–6 m	C1b-c (19); D1 (38); Clb-c/D1 (3)
	Pocillopora capitata	1–6 m	C1b-c (9); D1 (36); C1b-c/D1 (2)
	Pocillopora damicornis	1–6 m	C1b-c (15); D1 (35); C1b-c/D1 (1)
	Pocillopora meandrina	1–6 m	C1b-c (5); D1 (23); C1b-c/D1 (1)
Poritidae			
	Porites panamensis	0.5–1 m	C66 (10); C66a (13); C66b (2)
		0.5–14 m	C1 (18)
		10–14 m	C75 (2)
Siderastreidae			
	Psammocora brighami	10–14 m	C1f (2)
	Psammocora profundacella	2–7 m	C1f (5)
	Psamocora stellata	4–14 m	C1f (6)
	Psammocora superficialis	2–14 m	C1f (5)
Actiniaria			
	Isoaulactinia (=Bunodactis)	0.0–0.5 m	C66 (3)
	Aiptasia sp.	0.5 m	B1 (3)
Zoanthidea			
	Palythoa sp.	1–2 m	C1 (3)
	Protopalythoa sp.	2–10 m	C1o (2)
	Zoanthus pacificus	0.0–0.5 m	C29 (2); C29/A12 (1); C66 (1)

symbionts. To examine the sensitivity of these reactions, analyses were conducted on artificial mixtures as described in Materials and methods. Symbiont C1b-c was repeatedly detected at proportions as low as 0.2–1% of the total population by PCR-DGGE analyses of the ITS 1 and 2 (Fig. 5). Most *Pocillopora* colonies were scored as having only D1. If C1b-c were present in these samples, their abundance would have to be exceedingly low. Conversely, D1 cells in proportions below 50–35% of the total symbiont population were not detected by PCR-DGGE ITS 1 fingerprinting. ITS 2 analyses were slightly more sensitive and detected D1 when it comprised 10–30% of the total symbiont population (Fig. 5). Real-time PCR optimized to improve detecting minority populations of each symbiont supported the PCR-DGGE findings that most colonies hosted high proportions of one or the other symbiont (LaJeunesse et al. 2007). Results from real-time PCR suggest the possibility that genomes of clade C *Symbiodinium* have an rDNA copy number many times greater than that of clade D *Symbiodinium* and explains the bias of PCR-DGGE for clade C (unpubl. data).

The spatial distribution of colonies occupied by either *Symbiodinium* C1b-c or D1, the "C holobiont" and "D holobiont," appeared random and/or was patchy. The proportions of each holobiont were similar among *Pocillopora verrucosa*, *Pocillopora meandrina*, *Pocillopora capitata,* and *Pocillopora damicornis* (Table 2). Likewise, the relative prevalence of each *Pocillopora* holobiont remained similar in communities surveyed in 2004 and then in 2006 (Table 2). Initially, a statistical difference in the proportion of colonies with D1 was observed between three mainland and two island locations surveyed in 2004. Combining results from the island sites of Espiritu Santo Island and La Gaviota Island ($n = 22$; 12 D1, 10 Cb-c) and comparing it to mainland sites of Punta Arenas and Punta Galeras (32 D1 vs. 10 C1b-c), resulted in a significant difference in the prevalence of D1 colonies versus C1b-c colonies between habitats ($\chi^2 = 6.47$, $p < 0.01$; 1 df). However, subsequent analyses on a greater number of samples collected in 2006 from transects at Punta Galeras and La Gaviota Island showed no statistical differences in the proportion of D1 and C1b-c colonies between sites ($p > 0.05$) (Fig. 6). Preliminary surveys, however, suggest that *Pocillopora* spp. communities may lack colonies with Clb-c in protected shallow (1–2 m) bays (unpubl. data). Future comparisons between the southern Gulf of California with other regions of the eastern Pacific should address the possibility that partner combinations in these corals conform to larger scale environmental patterns.

Holobionts of *Porites panamensis* comprised several *Symbiodinium* spp. in clade C. Unlike *Pocillopora*, their distributions corresponded well to water temperature and/or depth. Three closely related types, C66, C66a, and C66b (LaJeunesse 2004), were found in specimens collected at depths less than 1 m. These were spatially separated from site to site (C66b from Espiritu Santu Island, C66 from

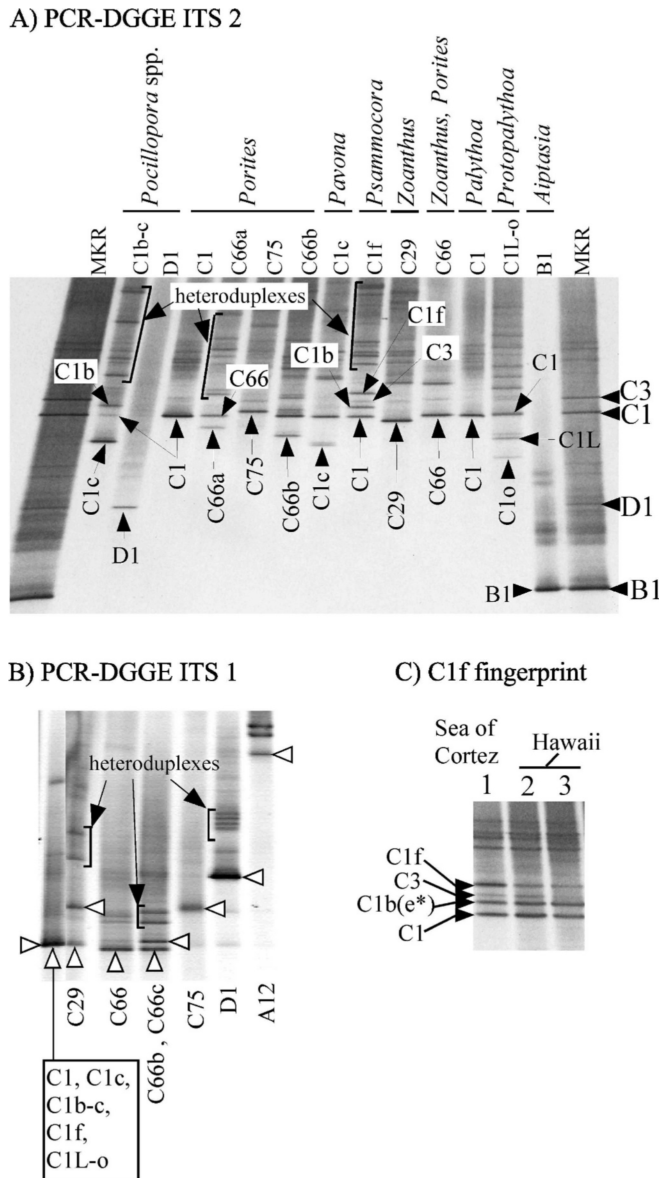

Fig. 2. Representative PCR-DGGE rDNA fingerprints of *Symbiodinium* spp. from cnidarians collected near La Paz. (A) Analyses of ITS 2 rDNA. The host taxonomic names of colonies where each fingerprint "type" was recovered are provided. Closed arrows signify bands excised and sequenced. (B) Similar analysis of ITS 1 rDNA. Fewer ITS 1 fingerprints could be characterized through the sequencing of diagnostic dominant or co-dominant bands (open arrows). The ITS 2 alphanumeric name that corresponds to each ITS 1fingerprint is given at the bottom of each lane. (C) The band intensities of "C1f" fingerprints are subtly different between samples acquired from *Psammocora* spp. in the Sea of Cortez (lane 1) and Hawaii (lane 2). In Hawaii, *Symbiodinium* C1f also occurs in *Leptastrea* (lane 3), *Fungia*, and *Cyphastrea*. *Band "b," erroneously called "e" in LaJeunesse et al. (2004*a*), is also part of the clade C *Symbiodinium* fingerprint (C1c-b) in *Pocillopora*.

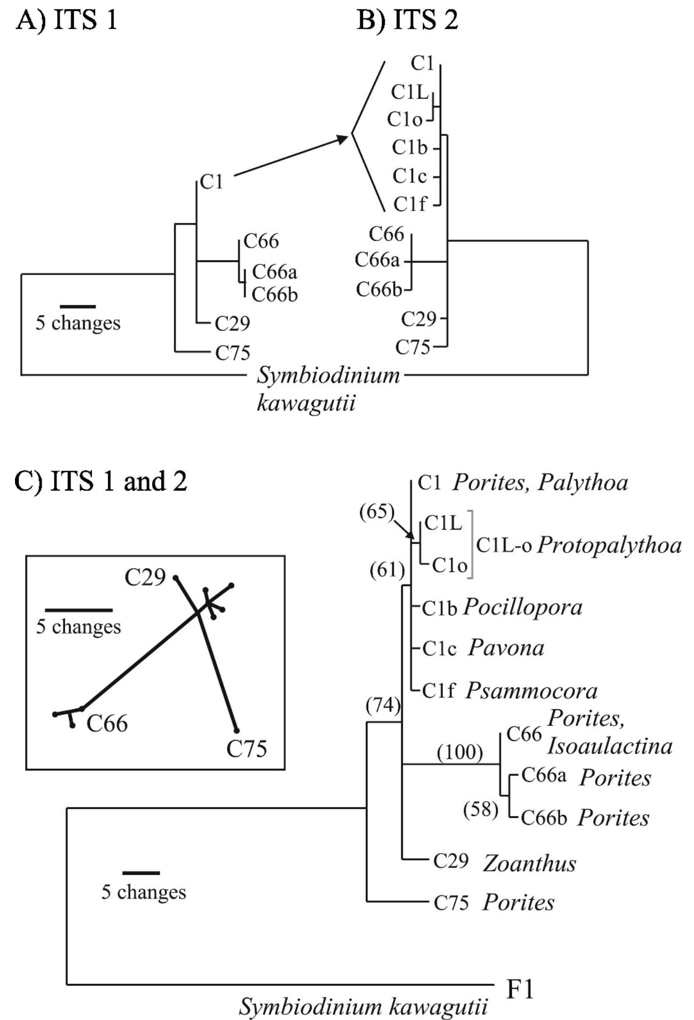

Fig. 3. Phylogenetic reconstructions, using maximum parsimony, of clade C *Symbiodinium* spp. (A) Phylogeny based on rDNA ITS 1 sequences generated from PCR-DGGE fingerprinting of rDNA.. Each sequence should represent the dominant intragenomic copy and is representative of an ecologically distinctive *Symbiodinium* population (LaJeunesse and Pinzon 2007). (B) Phylogeny based on ITS 2 sequences generated in the same manner. (C) Phylogeny based on the combined ITS 1 and ITS 2 sequences. Bootstrap values greater than 50%, based on 1,000 replicates, are in parentheses.

Punta Galaras, C66a from La Gaviota). Colonies hosting type C1, a symbiont not known to associate with *Porites* in other regions of the Pacific (LaJeunesse 2004), occurred at all locations typically below 1 m and down to a depth of 12 m. Type C75 was found only in colonies sampled below 5 m. Variable and often turbid water conditions attenuate the photic zone to such an extent that 5 m in the Eastern Pacific is almost equivalent to 15 m or 20 m in the western Pacific (Glynn and Ault 2000). The possibility that C75 is a low light-adapted symbiont, as its depth distribution suggests, requires physiological examination.

Discussion

Throughout much of the Indo-Pacific, knowledge remains limited about the diversity and biogeography of *Symbiodinium* spp. and about their associations with corals and other host cnidarians. However, there are indications of regional differences in symbiont diversity and host–

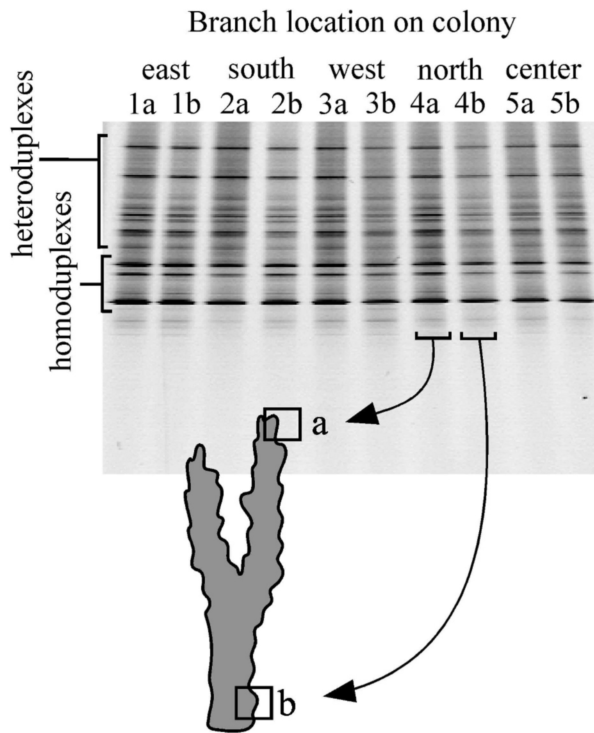

Fig. 4. Homogeneity found among *Symbiodinium* populations within *Pocillopora* colonies. A gel image showing one colony homogenous for C1b-c. Branches taken from the east, south, west, north, and center of 18 different colonies were analyzed. For each branch, sections from the tip and base were sampled. Each lane number corresponds to a particular branch with "a" designations referring to branch tips and "b" designations corresponding to sampling from the base of a branch. The C1b-c fingerprint consists of homoduplexes, comprising several intragenomic variants similar in sequence, and heteroduplexes that are common PCR-DGGE artifacts created by mispairings of single-stranded DNA molecules that differ by one or two nucleotide changes. The combined homoduplex and heteroduplex pattern is highly repeatable and diagnostic of this *Symbiodinium* taxon.

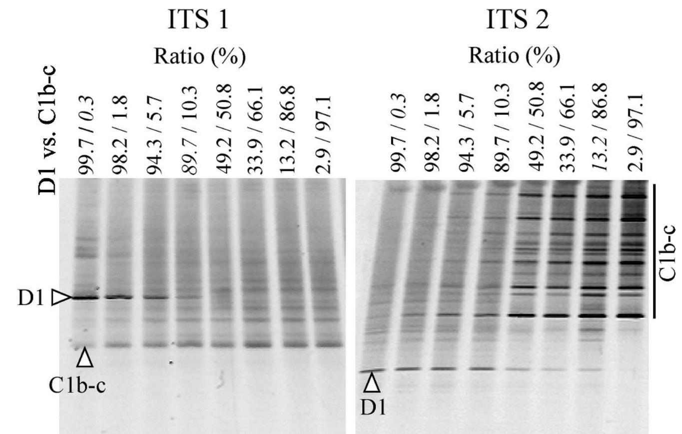

Fig. 5. Limitations for detecting mixed proportions of D1 and C1b-c using PCR-DGGE. The image represents one of three trials based on artificial combinations generated from freshly isolated *Symbiodinium*. Proportions are given as a percentage of the total population. Italicized percentages indicate the lowest proportions where the minority species is still resolved. Background populations of C1b-c as low as 0.2% were routinely detected, whereas populations of D1 cells at greater than 10% to 20% of the total sample were necessary for their detection. For most colonies dominated by D1 (Table 2), no background populations of C1b-c were detected.

symbiont combinations (Loh et al. 2001; LaJeunesse et al. 2004*a*; 2004*b*). The characterization of coral–algal associations from regions with unique physical-environmental conditions like the Gulf of California in the eastern Pacific contributes to a body of knowledge that may someday help explain how biotic and abiotic factors influence the evolution of host–symbiont partnerships.

Symbiodinium *diversity and host specificity*—Despite variable conditions of turbidity and exposure to seasonal upwelling of cool, nutrient-rich waters, coral–algal symbioses in the southern Gulf of California were highly specific. The majority of zooxanthellate cnidarians in the eastern Pacific are broadcast spawners and must acquire *Symbiodinium* spp. from environmental pools (Richmond and Hunter 1990). Hosts relying on horizontal symbiont acquisition (e.g., *Pavona*, *Psammocora*) harbored populations of *Symbiodinium* not found in other host taxa (Table 1). As a result, the host community in the Gulf of California lacked a host–generalist symbiont (LaJeunesse et al. 2004*b*). These findings support earlier indications that the host cell environment appears to be more important than the external environment as an axis of niche diversification among *Symbiodinium* spp. (Iglesias-Prieto and Trench 1997; LaJeunesse 2004).

Comparing assemblages of host-specific and/or rare symbionts may indicate the extent to which regions are geographically connected or isolated (LaJeunesse et al. 2004*a*; LaJeunesse 2004). Similar to other Pacific cnidarian communities, *Symbiodinium* diversity in the Gulf comprised mainly species in clade C (LaJeunesse et al. 2004*a*,*b*). Many of the *Symbiodinium* spp. identified can be found elsewhere in the Pacific (e.g., Fig. 2C), whereas some (e.g., C75, A12) may be endemic. The presence of C29 in *Zoanthus* sp. and C1f in *Psammocora* provides evidence for historical connections between the Gulf of California and central Pacific (Fig. 2C; LaJeunesse et al. 2004*a*,*b*). However, nowhere in the Indo-Pacific has *Porites* been documented to host C1, C66 (a and b variants), and C75 (LaJeunesse 2004). Their presence in this region indicates that long-term isolation under certain environmental pressures may contribute to the evolution of novel and geographically distinctive symbiotic combinations. The presence of a particular kind of symbiont, or host–symbiont combination, may someday be used to indicate geographic connectivity and/or isolation (Santos et al. 2004).

Variation among Pocillopora *symbioses: A case study in the coexistence of different holobionts*—In the eastern Pacific, *Pocillopora* colonies occupy a large percentage of available space in areas were coral communities are established (Glynn and Ault 2000). These ecologically dominant species appear to maintain remarkably stable associations with two kinds of *Symbiodinium*, D1 and C1b-c

Table 2. The prevalence of D1 and C1b-c *Pocillopora* holobionts in the Gulf of California near La Paz, Mexico. Numbers and percentages are given for holobionts of *Pocillopora* spp. calculated in 2004 and 2006. Numbers and percentages of holobionts determined for each species of *Pocillopora* (both years combined).

Host genus or species	Total colonies sampled	D1 holobionts	C1b-c holobionts	Mixed holobiont	% D1 holobiont	% C1b-c holobiont	% Mixed holobiont
By genus							
Pocillopora spp. 2004	65	44	20	1	67.7	30.8	1.5
Pocillopora spp. 2006	122	88	28	6	72.1	23.0	6.8
By species (2004 and 2006)							
Pocillopora verrucosa	60	38	19	3	63.3	31.7	5.0
P. meandrina	29	23	5	1	79.3	17.2	3.4
P. capitata	47	36	9	2	76.6	19.1	4.3
P. damicornis	51	35	15	1	68.6	29.4	2.0
Total colonies surveyed	187	132	48	7	70.6	25.7	5.3

(Tables 1, 2). Because the physiology of a coral colony is influenced by the physiology of the resident endosymbionts (Iglesias-Prieto et al. 2004; Rowan 2004; Berkelmens and van Oppen 2006) the ecological distributions of these combinations were examined in some detail at different locations in the Gulf of California and within individual colonies.

Rowan (2004) observed clear differences in habitat distributions between *Pocillopora* holobionts D and C living in Guam. For *Pocillopora* spp. in the Gulf, the external physical environment does not appear to be important in regulating the presence and/or competitive interactions between *Symbiodinium* spp C1b-c and D1 in hospite. At the colony level, symbiont populations were relatively uniform (as resolved by PCR-DGGE), and independent of the external light field (Fig. 4). The detection of both symbionts co-dominating a sample of tissue was rare (~ 5%) and did not correspond on any clear external physical factor including host pigmentation, position on the branch of a colony, and/or colony depth. Sometimes ''variegated'' colonies were found to contain isolated branches occupied by one or the other *Symbiodinium* sp. (LaJeunesse et al. 2007). Interestingly, results from monitoring symbiont populations in tagged colonies during recovery after a spring bleaching event indicated that colony ''shuffling'' between symbiont species does not seem to occur readily (LaJeunesse et al. 2007). These observations raise questions about the ecological and/or molecular-cellular basis for the temporal and spatial stability of homogenous and heterogeneous symbiont populations.

Different holobiont combinations likely possess ecological and physiological trade-offs (Iglesias-Prieto et al. 2004; Little et al. 2004). *Pocillopora* produce eggs containing symbionts acquired directly from the parent (vertical symbiont acquisition; Glynn et al. 1991). Therefore a difference in the proportion of colonies harboring D1 versus C1b-c may correspond to the present ecological success of each combination (i.e., holobiont competition and/or differential survival). Terrestrial runoff, rapid temperature changes due to upwelling, and wide shifts in

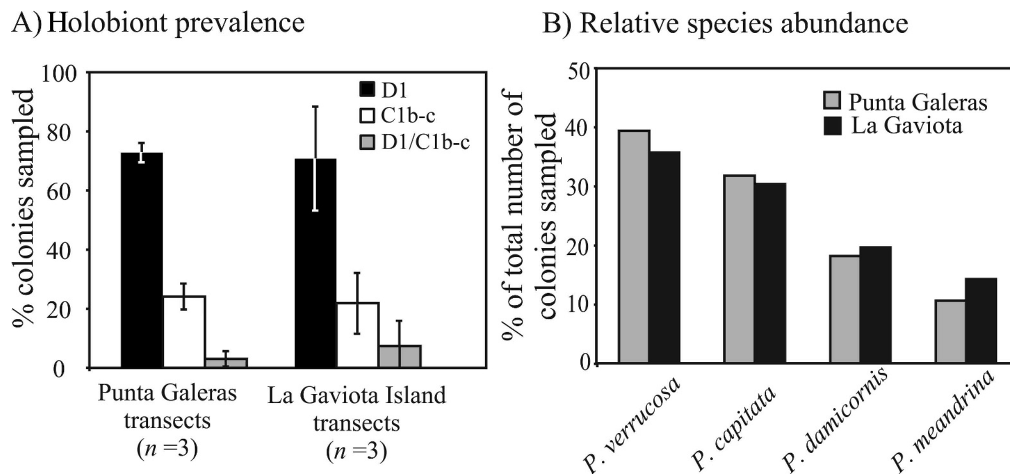

Fig. 6. (A) Variability in the frequency of D1 versus C1b-c holobionts of *Pocillopora* spp. along transects (n = 3, an average of 20 colonies per transect, SD ± 2) at two locations, Punta Galeras and La Gaviota Island (error bars, ± SD). (B) Similar compositions of *Pocillopora* spp. were unintentionally sampled at each location, indicating a similar host community composition (numbers were totaled from each of the three transects).

seasonal irradiance may favor the ecological prevalence of one holobiont combination over the other one. Higher summer water temperatures in protected shallow bays may explain the prevalence of colonies hosting D1 at these particular locations (> 95%, unpubl. data). The last significant bleaching event to affect this area was in 1998, when approximately 30% of colonies bleached (Reyes-Bonilla 2001), a figure roughly equivalent to the proportion of colonies hosting C1b-c. The persistence of the C holobiont, however, indicates that this combination tolerates environmental conditions in the region.

Natural selection among holobiont variants is probably significant during episodes of severe stress. *Pocillopora* holobionts involving clade C and clade D *Symbiodnium*, found in Panama (Glynn et al. 2001), each responded differently to thermal stress during the 1997–1998 ENSO-induced beaching event. During the height of bleaching, visually pale colonies all contained C *Symbiodinium*, whereas unbleached "healthy-looking" colonies contained clade D (presumably D1). Differential mortality of colonies harboring clade C probably explains the increase in the proportion of *Pocillopora* spp. colonies harboring clade D *Symbiodinium* from ~26% in 1995 to ~61% in 2001 (Glynn et al. 2001). Although the survivorship among sampled colonies was not followed, and therefore the increase in D holobionts in relation to mass bleaching and mortality remains correlative, community-wide mortality among *Pocillopora damicornis* and *Pocillopora elegans* (= *verrucosa*) at the Uva Island site was approximately 5% and 50%, respectively (Glynn et al. 2001). It should be noted that a >30% variance in D holobionts occurred at Punta Galeras from transect to transect (Fig. 6A). Such variability and patchiness among proportions of *Pocillopora* holobionts may limit the interpretations of Glynn et al. (2001) and Baker et al. (2004).

The clear specificity and stability between clade D *Symbiodinium* and *Pocillopora* spp. (Table 1) suggests that this is a naturally evolved association, as it is for other coral taxa in different regions around the Pacific (Chen et al. 2003). Although bleaching and mortality events may increase the prevalence of this combination, the high proportion of D holobionts at higher eastern Pacific latitudes is probably not the result of coral bleaching (Baker et al. 2004). The wide fluctuation in nutrients, temperature, and turbidity present in the eastern Pacific, Taiwan (Chen et al. 2003), and the Arabian Gulf (Sheppard 1992) correlate with many symbioses involving clade D *Symbiodinium* (c.f., Toller et al. 2001; Ulstrup and Van Oppen 2003; Baker et al. 2004). Understanding the photophysiology of these symbionts may lend insight into why their symbioses commonly occur in especially variable environments.

The evolution of regionally distinctive host–symbiont combinations around the world are influenced in part by the environment, prolonged isolation, and the community composition of host and symbiont taxa (Thompson 1994). Although isolated from the rest of the Pacific, coral–algal associations in the Gulf of California were similar in cladal composition (dominated by clade C *Symbiodinium*). However, levels of host–symbiont specificity appeared to be greater than in the western Pacific, where host diversity is considerably higher and environmental conditions are relatively stable. The finding of unusual symbiotic combinations in this region, especially for *Porites*, offers examples of how specificity between host and symbiont populations might change during long-term shifts in the global environment (Baker 2003; LaJeunesse 2004).

Over evolutionary time, coral–algal symbioses appear to have undergone considerable rearrangement in partner combinations that were probably facilitated by major episodes of global or regional climate change (Rowan and Powers 1991; LaJeunesse 2004). Questions remain as to whether colonies hosting thermally sensitive symbionts will be eliminated through natural selection over evolutionary time scales or whether short episodes of severe stress, acting at ecological time scales, will initiate the acquisition and/or establishment of another, and perhaps novel, symbiont population in colonies that survive (Buddemeier and Fautin 1993; Baker 2001, LaJeunesse et al. 2004*a*; LaJeunesse 2004).

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